LETTER TO THE EDITOR

Calcium Release from the Sarcoplasmic Reticulum

Dear Sir:

Recently, Melzer et al. (1–3) have described a procedure for determining the rate of Ca^{++} release from the sarcoplasmic reticulum (SR), based on the Ca^{++} transients obtained in frog skeletal muscle fibers under voltage clamp conditions. The authors and the field editor have asked me to comment on these papers, since my view differs from that of the authors with respect to a number of important issues.

In this communication I wish to justify in some detail (*a*) why the approach used by the authors does not lead to the determination of the actual rate of Ca^{++} release from the SR into the sarcoplasm and (*b*) why the authors cannot extract from their data the true rate of Ca^{++} release from the SR into the sarcoplasm, without their knowing the kinetics of Ca^{++} movements associated with fast and slow Ca^{++} binding sites in the sarcoplasm and the rate of Ca^{++} removal from the sarcoplasm by the SR.

From their experiments the authors can determine, at any moment, the ionized Ca concentration and dCa^{++}/dt in the preparation. Furthermore, dCa^{++}/dt can be defined by the general expression as:

$$dCa^{++}/dt = (dCa/dt)_{\substack{\text{SR release into the} \\ \text{ionized Ca compartment}}} - (dCa/dt)_{\substack{\text{removal from the} \\ \text{ionized Ca compartment}}} . \quad (1)$$

The removal system of Ca^{++} from the ionized Ca compartment comprises the SR and the Ca^{++}-binding sites in the sarcoplasm which display both fast and slower kinetics (4). All Ca^{++} movements associated with the Ca^{++} removal system are dependent on the ionized Ca in the sarcoplasm and on time. Therefore, they must be continuous functions of time for the same reasons as those mentioned by the authors in their last paper (3). If, as the authors assume, the sarcoplasmic reticulum suddenly ceases to release Ca^{++} into the ionized Ca compartment at the end of a depolarizing pulse, then based on Eq. 1 one can determine the net rate of Ca^{++} removal from the ionized Ca compartment at that moment and the rate of Ca^{++} release from the SR can be calculated, as the authors propose.

The inspection of the traces representing dCa^{++}/dt at the end of the depolarization pulses (Fig. 4 in reference 3), however, clearly indicates that the SR does not suddenly cease to release Ca^{++} into the sarcoplasm, but continues to release Ca^{++} for some time after the repolarization of the sarcolemma. Therefore, the contribution of the net Ca^{++} removal systems from the ionized Ca compartment (from the arbitrary moment when the SR is assumed to have stopped releasing Ca^{++}, to the moment when the depolarization has terminated) must be extrapolated back in time. This extrapolation cannot be made simply, with any degree of certainty, unless the kinetics of the main groups of Ca^{++}-binding sites in the sarcoplasm are precisely known.

To illustrate this point in Fig. 1, I have represented the time course of the individual Ca^{++} movements associated with two

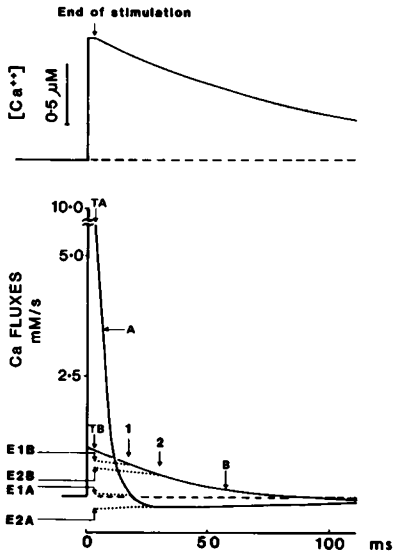

FIGURE 1 Ca^{++} fluxes (lower traces *A* and *B*) associated with two types of Ca^{++}-binding sites after a change in $[Ca^{++}]$ (upper trace) similar to that observed in single skeletal muscle fibers (see text). The assumed total concentration of both types of sites was 0.1 mM. Both types of sites were assumed to have an affinity for Ca^{++} of $10^6 M^{-1}$, but the rate of release of bound Ca^{++} was different ($100\ s^{-1}$ for trace *A* and $10\ s^{-1}$ for trace *B*). At time 0 all Ca^{++}-binding sites were assumed to exist in free form. The level of Ca^{++} fluxes indicated by the arrows *E1A*, *E1B*, *E2A*, and *E2B* were obtained by extrapolating the Ca^{++} fluxes at the time marked by the arrows *1* and *2*, respectively, to the end of stimulation. The assumption made for this back extrapolation was that the contribution of the Ca^{++}-binding sites to the net rate of Ca removal from the ionized Ca compartment was proportional to the free Ca concentration over this period of time (3). The dashed lines represent the baseline for the Ca^{++}-transient (upper part) and the zero line for the Ca^{++} fluxes. Ca uptake from the environment is considered to be positive.

classes of Ca^{++}-binding sites when the Ca^{++}-transient (upper trace in Fig. 1) had a time course similar to that observed by Melzer et al. (1–3). The Ca^{++}-transient was assumed to start with a sudden rise in $[Ca^{++}]$ to a plateau which continued with an exponential decay immediately after the sudden repolarization of the membrane. The lower traces (*A* and *B*) in Fig. 1 show the Ca^{++} fluxes associated with two types of Ca^{++}-binding sites of similar affinity to Ca^{++} but of different kinetics. The important point to note is that the time courses of such Ca^{++} fluxes are not monotonous functions of time after membrane repolarization. The points marked by the arrows *E1A* and *E1B* in Fig. 1 are the extrapolated values from 14 ms (an arbitrary value used in reference 3) after the end of the stimulation to the moment of sudden repolarization of the membrane, using the method employed by the authors (Method 3, reference 3). Clearly, the extrapolated values are quite different from the true values *TA* and *TB*, marked on the lower traces in Fig. 1. With such

extrapolation procedures, the errors in estimating the contribution of the various Ca^{++}-binding sites in the sarcoplasm to the net rate of Ca^{++} removal from the ionized Ca compartment increase with the length of time that has elapsed from the end of stimulation (compare the points *E2A* with *E1A* and *E1B* with *E2B*). This means that the errors will be larger if the SR is assumed to have stopped at a later moment, say 50, 100, or 200 ms after repolarization. The magnitude of the errors caused by extrapolation also depends largely on the rate of $[Ca^{++}]$ decrease after the repolarization of the membrane and on how far from or how close to the steady state the various Ca^{++}-binding sites and the ionized Ca are at the end of the depolarizing pulse. Therefore, the type of analysis based on Eq. 1 to deduce the time course of the SR–Ca^{++}-release component cannot be of much practical use unless some firm assumptions about the kinetics of all known Ca^{++}-binding sites in the sarcoplasm are known.

Initially, the authors considered only instantaneous Ca^{++} equilibrating sites in the sarcoplasm and ignored known Ca^{++}-binding sites of fast (but not instantaneous) and slower kinetics (1). Subsequently they also considered some fast Ca^{++}-binding sites but continued to ignore the contribution of the known slower Ca^{++}-binding sites (2, 3). In fact the authors clearly state in their discussion that they could not accommodate the existence of Ca^{++}-binding sites of slower kinetics in their representation of sarcoplasmic Ca^{++} movements. If these sites were also considered, as suggested by Lüttgau and Stephenson (5), then the predicted time course of the total Ca in the sarcoplasm and that of the SR–Ca^{++}-release component would have changed markedly from that proposed by the authors in their papers. Thus, the time course of the Ca^{++}-release from the SR would follow the time course of the actual Ca^{++}-transient more closely, as indicated earlier (6–8) and it would not be possible to assume that the Ca^{++}-release from the SR ceases immediately after the repolarization of the membrane.

In conclusion, there is no simple way to determine the time course of the Ca^{++}-release component from the sarcoplasmic reticulum correctly unless one considers not only the contribution of the instantaneous and fast Ca^{++}-binding sites in the sarcoplasm, but also the contribution of the other individual components that determine the Ca^{++}-transient in skeletal muscle.

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